



# Characterization in vitro and in vivo of the oxygen dependence of an enzyme/polymer biosensor for monitoring brain glucose

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## Abstract

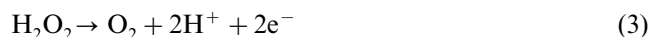
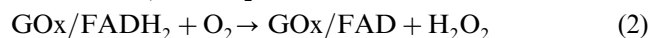
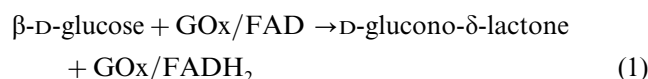
The oxygen dependence of a first generation amperometric biosensor was investigated in vitro and in vivo by monitoring its glucose response as a function of solution pO<sub>2</sub>. The biosensor was a glucose oxidase (GOx) modified poly(*o*-phenylenediamine) coated Pt cylinder electrode (Pt/PPD/GOx) that has been designed for neurochemical analysis in vivo. Two types of oxygen probes were used: a self-calibrating commercial macroelectrode in vitro; and a carbon paste microelectrode in vivo. Calibrations in vitro showed that oxygen interference in the operation of Pt/PPD/GOx electrodes was minimal for concentrations of glucose (~0.5 mM) and oxygen (~50 μM) found in brain ECF. This observation was confirmed by simultaneous monitoring in vivo of brain glucose and oxygen in the awake rat. However, at levels of glucose normally found in peripheral tissues (~5 mM), the oxygen dependence was severe. We conclude that the oxygen sensitivity of Pt/PPD/GOx biosensors does not preclude their reliable use in media containing low glucose levels, such as brain ECF. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Poly(*o*-phenylenediamine); Enzyme modified electrode; Amperometry; Voltammetry in vivo; Glucose oxidase; Electropolymerization

## 1. Introduction

The incorporation of biologically active molecules into sensing devices has promoted considerable development of fast, efficient and inexpensive assays for a wide range of analytes in research, clinical, industrial and environmental applications (Braguglia, 1998; Zhang et al., 2000; Karube and Nomura, 2000; Liang et al., 2000; Wang, 2001). In particular, the immobilization of enzymes onto electrode surfaces provides biosensors that can be categorized broadly as first, second or third generation devices depending on the mode of electron transfer between the redox center of the enzyme and the electrode surface (Wang, 1993; Scheller et al., 1993). Using the flavoenzyme glucose oxidase (GOx), the majority of these sensors have focused on the amperometric detection of glucose, both as a model system and because of the importance of glucose as a target analyte (Magner, 1998).

The overall enzymatic process, that is similar for many oxidases, may be written as Eq. (1) and Eq. (2):



where FAD is the oxidised form of the prosthetic group, flavin adenine dinucleotide. Eq. (3) represents the electrochemical step, generating current, and is generally carried out amperometrically at relatively high applied potentials. A clear limitation of this reaction scheme (1–3) from an analytical perspective, is that the signal (Eq. (3)) may be prone to interference by the effect of changes in pO<sub>2</sub> in Eq. (2), undermining its use to reflect glucose concentrations unambiguously. Indeed, in some situations, such as bioprocess monitoring and in vivo measurements, O<sub>2</sub> availability may be very limited. Attempts to overcome this problem include the use of membranes (Sakamoto, 1992) and different electrode geometries (Lucisano and Gough, 1988; Pishko et al., 1991), and while many of these modifications have helped alleviate the oxygen problem, they have not succeeded in eliminating it.

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Classical, or first generation, devices involve monitoring either the consumption of  $O_2$  (Clark and Lyons, 1962) or the formation of  $H_2O_2$  (Updike and Hicks, 1967) by the oxidase. The latter remains the most common form of biosensor despite requiring a large overpotential at which reducing agents present in the sample can contribute to the faradaic current. Although some of the problems associated with anodic  $H_2O_2$  detection have been minimized by using electrocatalytic  $H_2O_2$  reduction at low potentials (Gao et al., 1992; Vreeke et al., 1992; Wang et al., 1994, 1996), the question of sensitivity to  $pO_2$  appears in an additional form—the possibility of direct faradaic reduction of  $O_2$ .

As an alternative approach, there has been an upsurge in the development of second generation systems in which  $O_2$  is replaced with an artificial mediator. Many such redox mediators have been used successfully, including metallocenes, quinones, ferrocyanide and tetrathiafulvalene (Di Gleria et al., 1986; Wang, 1993). The advantages of such devices are that the electrode may be operated at lower applied anodic potentials, and since molecular oxygen is not the final electron acceptor, these sensors might be expected to be relatively independent of changes in  $pO_2$ . There are reports, however, where artificial mediators could not compete effectively with  $O_2$  (Razumas et al., 1993; Wilke and Muller, 1994; Martens and Hall, 1994), and the continuous leaching of potentially toxic mediator is an inherent problem for applications in vivo.

A number of laboratories have described the development and characterization of a first generation glucose sensor based on a GOx-modified poly(*o*-phenylenediamine) coated Pt (Pt/PPD/GOx) electrode that possesses a variety of properties indicating suitability for neurochemical applications (Sasso et al., 1990; Malitesta et al., 1990; Reynolds and Yacynych, 1993; Bartlett and Birkin, 1994; Lowry et al., 1994a). These attributes include fast response time, linearity over the relevant concentration range, effective elimination of interference by endogenous reducing agents such as ascorbic acid, freedom from protein and lipid fouling, stability in vivo, and ease of miniaturisation (O'Neill and Lowry, 2000). In a preliminary publication, we demonstrated that Pt/PPD/GOx sensors might also be free of  $O_2$  interference in applications both in vitro and in vivo by showing that they responded to glucose with similar sensitivity over a wide range of  $pO_2$  values in vitro (Lowry et al., 1994b). As this phenomenon has important implications both for the development of biosensors and for their operation in biological systems, where  $O_2$  availability is restricted, we examine it now in more detail. A preliminary report of some of these findings has been published (Dixon et al., 2001).

## 2. Materials and methods

### 2.1. Chemicals and solutions

The enzymes GOx from *Aspergillus niger* (EC 1.1.3.4, type VII-S), Ketamine and acetazolamide (Diamox) were obtained from Sigma Chemical Co. *o*-Phenylenediamine (*o*-PD) (1,2-diaminobenzene, Sigma),  $\alpha$ -D-(+)-glucose (Sigma), L-ascorbic acid (AA, BDH Biochemical grade), and bovine serum albumin (BSA, Armour Pharmaceutical Co. Ltd, fraction V from bovine plasma) were used as supplied. Carbon paste was prepared by thoroughly mixing 2.83 g carbon powder (UCP-1-M, Ultra Carbon Corporation, Bay City, MI) and 1.0 ml silicone oil (Aldrich, Cat. No. 17 563-3).

A stock 1 M solution of glucose was prepared in water, left for 24 h at room temperature to allow equilibration of the anomers, and then stored at 4 °C. All experiments in vitro were carried out in a phosphate buffered saline (PBS) solution, pH 7.4; NaCl (Merck, BP USP grade, 150 mM),  $NaH_2PO_4$  (BDH, AnalaR grade, 40 mM) and NaOH (BDH, GPR grade, 40 mM). A 200 U/ml solution of GOx was prepared by dissolving 15 mg in 10 ml of PBS. The 300 mM *o*-PD monomer solution was prepared using 0.32 g of *o*-PD and 50 mg of BSA in 10 ml of PBS and sonicating at 25 °C for 30 min.

### 2.2. Instrumentation and software

Experiments were microcomputer controlled with data collection accomplished using either a Biodata Microlink interface or a National Instruments (NI, Austin, TX) AT-MIO-16 data acquisition board linked to a low-noise, low-damping potentiostat (Biostat II, Electrochemical and Medical Systems, Newbury, UK). The 2-channel configuration of the potentiostat allowed different potential waveforms to be applied to two working electrodes simultaneously. In-house software was written in QuickBASIC (version 4.0) and NI LabWindows (version 2.1) QuickBASIC environments to perform all amperometric experiments and to collect, plot, and do a preliminary analysis of the data.

### 2.3. Working electrode preparation

The fabrication of the working electrode biosensors has been described in detail recently (O'Neill et al., 1998; Lowry et al., 1998b). Briefly, cylinders (~1 mm long) of freshly cut Teflon-insulated Pt wire (60  $\mu$ m radius) were dipped into a 200 U/ml solution of GOx to allow adsorption of the enzyme, and then removed to dry. The enzyme-coated wire was then immersed into PBS containing the monomer and BSA, and electropolymerisation carried out at 700 mV vs. saturated calomel

electrode (SCE) for 15 min to produce Pt/PPD/GOx sensors.

The carbon paste electrodes (CPEs) for the detection of solution  $O_2$  in vivo were made from Teflon-coated silver wire (200  $\mu\text{m}$  internal diameter, 280  $\mu\text{m}$  external diameter). The Teflon insulation was slid along the wire to create an approximately 1-mm deep cavity that was packed with carbon paste using a bare silver wire as plunger. When not in use all electrodes were stored in PBS at 4 °C.

A self-calibrating commercial membrane-covered amperometric oxygen sensor ( $\sim 1$  cm diameter) was used to quantify solution oxygen concentration in vitro. The model used was a Cellox 325 connected to an Oxi 340A meter (Wissenschaftlich-Technische Werkstätten GmbH from Carl Stuart Ltd, Dublin, Ireland), incorporating a temperature probe for automatic compensation. Reliable quantification of  $O_2$  using this device required constant stirring of the solution at a rate of  $\sim 2$  Hz. The sensor range was 0.0–199.9%  $O_2$  (100% corresponding to air saturation) with a resolution of 0.1%. For some purposes, this percentage was converted to an estimated concentration of  $O_2$ , taking 200  $\mu\text{M}$  to correspond to 100% (Bourdillon et al., 1982; Zhang and Wilson, 1993).

#### 2.4. Amperometric experiments in vitro

All experiments in vitro were performed in a standard three-electrode glass electrochemical cell containing 20 ml PBS thermostated at  $25.0 \pm 0.5$  °C. A SCE was used as the reference electrode, and a large silver wire, isolated in a compartment containing PBS, served as the auxiliary electrode. The applied potential for amperometric studies was +700 mV vs. SCE unless stated otherwise, which is the value generally used for  $H_2O_2$  detection. To avoid contamination by oxygen, the electrochemical cell was contained within an Atmosbag<sup>TM</sup> (Sigma), a two-hand 0.003-in. gauge polyethylene bag that was sealed and filled with  $N_2$  during experiments, inflating to a volume of 280 l.

The addition of glucose was not carried out until the electrodes were well settled giving a steady background in air-saturated PBS. After the addition of a single aliquot of glucose, the bag was sealed and high-grade  $N_2$  ( $< 5$  ppm  $O_2$ ) introduced. The time required to achieve minimal  $O_2$  concentrations in solution ( $< 1$   $\mu\text{M}$ ) was approximately 3 h (Fig. 1A), even at a driving pressure of 1  $\text{kg}/\text{cm}^2$  and an open nipple valve on the  $N_2$  cylinder. Oxygen sensor data and biosensor data were recorded simultaneously through the transition from air saturation to  $N_2$  saturation and/or vice versa, the biosensor data normalized relative to the respective air saturation value, and plotted against the measured concentration of  $O_2$ .

To calibrate the CPEs used for oxygen detection in vivo, differential pulse amperometry (DPA, one pulse

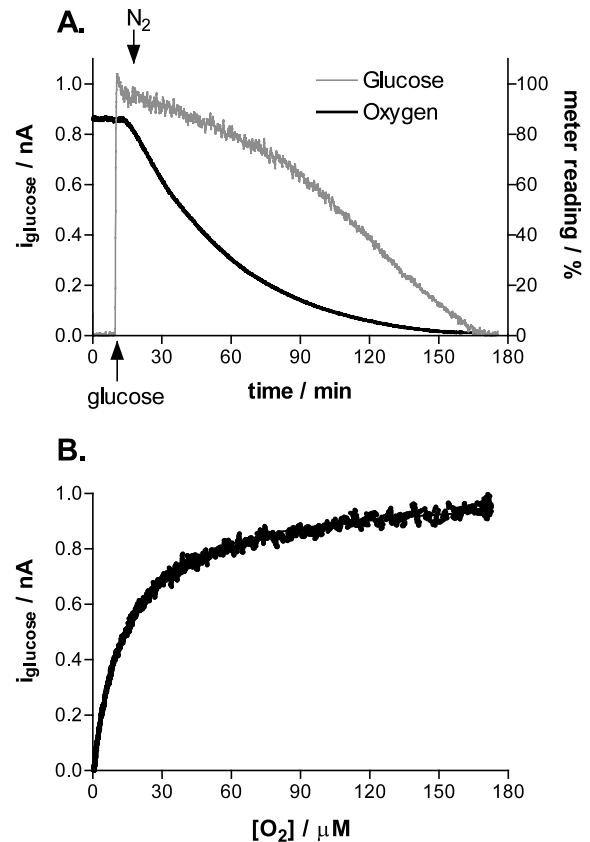


Fig. 1. (A) Example of raw data for oxygen (Cellox sensor, black, right axis) and glucose (Pt/PPD/GOx biosensor, grey, left axis) recorded in vitro. 0.5 mM glucose was injected into PBS, containing ambient levels of air, at the first arrow. Nitrogen was then introduced (second arrow) into the AtmosBag surrounding the electrochemical cell. Removal of  $O_2$  followed a one-phase exponential decay with a half-life of  $35 \pm 5$  min ( $n = 4$ ). (B) Glucose–oxygen correlation plot for data shown in A above. Non-linear regression using Eq. (4), gave  $K_m(O_2) = 15 \pm 1$   $\mu\text{M}$  and  $i_{\text{max}} = 1.0 \pm 0.1$  nA, with  $R^2 > 0.996$ .

pair:  $-150$  to  $-350$  mV and  $-350$  to  $-550$  mV from a resting potential of  $-150$  mV) was used in vitro as described recently (Lowry et al., 1996, 1997). The CPEs were calibrated after removal from the brain because implantation affects the sensitivity of these paste electrodes (O'Neill, 1993; Kane et al., 1998). Residual DPA current recorded in  $N_2$ -saturated PBS, due to uncompensated capacitance and surface redox processes, was subtracted from the current recorded in air-saturated (200  $\mu\text{M}$ ) and  $O_2$ -saturated (1.26 mM) buffer (Bourdillon et al., 1982; Zhang and Wilson, 1993).

Non-linear regression analysis of the oxygen dependence of the glucose signal was carried out using a software package (Prism, GraphPad Software, Inc., San Diego, CA) and a Michaelis–Menten type equation (Eq. (4), where  $i$  is the biosensor signal). The reactant, sub, is usually the enzyme substrate (glucose, Eq. (1)) or, as in this study, the co-substrate (dioxygen, Eq. (2)) whose concentration is being varied, and  $K_m$  is an apparent Michaelis–Menten constant. When sub is the target

analyte, a large value of  $K_m$  is beneficial ( $i$  is then linear in [sub]). However, when sub is a possible interference species, a small value of  $K_m$  is advantageous because  $i$  is then independent of [sub] except at very low [sub] values; Fig. 1B.

$$i = \frac{i_{\max}}{1 + \frac{K_{m,\text{sub}}}{[\text{sub}]}} \quad (4)$$

### 2.5. Experiments in vivo

Male Sprague–Dawley rats weighing 200–300 g were anaesthetised and implanted with two electrochemical sensors as described previously (Lowry and Fillenz, 1997). The Pt/PPD/GOx electrode was implanted in the right striatum at co-ordinates (skull levelled between bregma and lambda): A/P +1.0 from bregma, M/L +2.5, and D/V –5.0 (from dura). A CPE was implanted in the left striatum at corresponding co-ordinates. The reference electrode (8T silver wire, 200- $\mu\text{m}$  bare diameter) was placed in the cortex, the auxiliary electrode (8T silver wire) placed between the skull and dura, and an earth wire (8T silver wire) attached to one of the support screws. The electrodes were attached to a socket, and fixed to the skull with screws and dental acrylate. Animals were allowed 24 h to recover after surgery. All procedures were specifically licensed under the Animals (Scientific Procedures) Act, 1986 (UK) and involved simultaneous monitoring of  $\text{O}_2$  using DPA with the CPE (Lowry et al., 1996, 1997) and of glucose using amperometry with the Pt/PPD/GOx sensor held at +700 mV (Lowry et al., 1994a, 1998b).

All data are reported as mean  $\pm$  SEM with  $n$  = number of electrodes. The statistical significance of all drug effects was estimated using Student's two-tailed paired  $t$ -tests on the absolute currents.

## 3. Results and discussion

### 3.1. Oxygen detection in vitro

A recent report on the oxygen dependence of Pt/PPD/GOx biosensors (Wang and Lu, 1998) prompted us to re-appraise our previous conclusion, based on a preliminary pilot study (Lowry et al., 1994b), that these devices were essentially free of oxygen interference at physiologically relevant levels of  $\text{O}_2$ . The more rigorous quantification of solution oxygen achieved here, using the self-calibrating Cellox 325 electrode, showed that in our previous experimental design (cell open to the atmosphere), the solution oxygen concentration did not fall below  $36 \pm 5 \mu\text{M}$  ( $n=5$ ), even after extensive

bubbling of the PBS with nitrogen, and explains the discrepancies between the two reports. To confirm the ability of the commercial  $\text{O}_2$  sensor/meter system to quantify zero  $\text{pO}_2$  levels, 1 mM of the  $\text{O}_2$ -scavenger, sodium dithionite, was added to the  $\text{N}_2$ -challenged PBS. When the dithionite was added, the reading dropped to a value not significantly greater than zero ( $\sim 0.5 \mu\text{M}$   $\text{O}_2$ ).

To avoid this oxygen contamination, an AtmosBag was used to isolate the cell from the atmosphere in all subsequent experiments in vitro, and nitrogen gas introduced directly into the bag (not bubbled through the solution) to avoid turbulent convection. Fig. 1A shows the time course for removal of oxygen from the PBS and the effectiveness of the bag in preventing oxygen contamination in the cell. Removal of  $\text{O}_2$  had a half-life of  $35 \pm 5$  min ( $n=4$ ) and sub-micromolar levels of  $\text{O}_2$  could be achieved after  $\sim 3$ –4 h of flushing with nitrogen.

### 3.2. Oxygen-sensitivity of Pt/PPD/GOx responses to glucose in vitro

The glucose calibration profile and sensitivity of this type of enzyme/polymer-based sensor have been reported extensively (Sasso et al., 1990; Malitesta et al., 1990; Reynolds and Yacynych, 1993; Bartlett and Birkin, 1994; Lowry et al., 1994a). Because stirring was necessary in the present experiments to allow the Cellox  $\text{O}_2$  probe to function quantitatively, and because convection affects the glucose current (Bartlett and Whitaker, 1987), the absolute size of the glucose signal is neither comparable to previous studies nor of much relevance. The focus here is the effect of oxygen on the glucose response, and so a normalized current was calculated, using the air-saturated current for each glucose concentration as 100%.

Fig. 1A shows an example of the absolute current response of a Pt/PPD/GOx electrode to 0.5 mM glucose in air-saturated stirred PBS. When oxygen was then removed from the solution, both the  $\text{O}_2$  and biosensor signals decreased, albeit with different time courses, and approached zero as oxygen was completely removed from the system, as expected from Eqs. (2) and (3). Fig. 1B shows the glucose– $\text{O}_2$  correlation plot constructed from the raw data in Fig. 1A; the plot was hyperbolic (Eq. (4)) with  $K_m(\text{O}_2) = 15 \pm 1 \mu\text{M}$  and  $R^2 > 0.996$ . The 0.5 M glucose current, therefore, was effectively insensitive to changes in  $\text{O}_2$  levels above  $\sim 40 \mu\text{M}$   $\text{O}_2$ , but the sensitivity to  $\text{O}_2$  increased below this level.

The turnover of  $\text{O}_2$  in the polymer/enzyme composite membrane (Eq. (2)) depends on the rate at which glucose binds to the enzyme (Eq. (1)), i.e., on the concentration of glucose. We therefore carried out experiments similar to those in Fig. 1A over a range of glucose concentrations (0.5, 1.0, 2.5, 5.0, 7.5 and 10.0



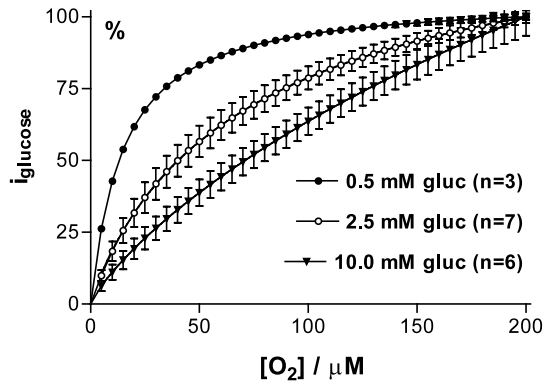


Fig. 2. Examples of averaged normalized glucose–oxygen correlation plots (Fig. 1) for three of the six concentrations of glucose studied over the relevant range of  $O_2$  concentration ( $N_2$  saturation to air saturation) showing the increased sensitivity of the biosensor signal to solution  $pO_2$  as the concentration of glucose was increased. Non-linear regression plots shown were obtained using Eq. (4). Averaged  $K_m$  values for the individual plots are presented in Fig. 3.

mM), and analysed the results as in Fig. 1B. For clarity, Fig. 2 shows only the glucose– $O_2$  correlation plots of averaged normalized data for 0.5, 2.5 and 10.0 mM glucose. As predicted, the  $O_2$  dependence became more acute at higher glucose concentrations. For 10 mM glucose, a level not much greater than the normoglycemic value in human blood (Eyre et al., 1994) and less than that observed under hyperglycemic diabetic conditions (Hofer and Lanier, 1991), the  $O_2$  dependence was close to maximal with a  $K_m(O_2) = 173 \pm 47 \mu M$  ( $n = 6$ ). The Pt/PPD/GOx sensor design would therefore not be suitable for monitoring blood glucose in vivo. Although the trend in  $K_m(O_2)$  from 0 to 10 mM glucose shown in Fig. 3A demonstrates a high  $O_2$  sensitivity even at moderate glucose levels, the question remained whether Pt/PPD/GOx electrodes might be suitable for monitoring brain glucose, where the estimated level is much lower than in blood. ECF glucose in rat striatum has been determined by both quantitative microdialysis:  $350 \pm 20 \mu M$  (Fray et al., 1997),  $350 \pm 16 \mu M$  (Lowry et al., 1998a) and  $710 \mu M$  (McNay et al., 2001) and biosensor data: 300–400  $\mu M$  (Lowry and Fillenz, 2001) in the awake rat.

Although  $O_2$  ECF levels farthest from blood brain capillaries can be as low as 5  $\mu M$  (Siesjo, 1978), electrodes the size of CPEs and Pt/PPD/GOx sensors sample the average tissue ECF concentration, estimated at  $\sim 50 \mu M$  (Nair et al., 1987; Murr et al., 1994). We therefore calculated the percentage  $O_2$  interference in the glucose signals at 50  $\mu M$   $O_2$  per micromolar change in  $O_2$  concentration ( $S_{\%}$ ), i.e., the tangential slope of plots similar to those in Fig. 1B determined at 50  $\mu M$   $O_2$ . The results of this analysis are shown in Fig. 3B. The data points were consistent with both linear (not shown) and hyperbolic fits that were distinguished only by their behaviour as the concentration of glucose approached

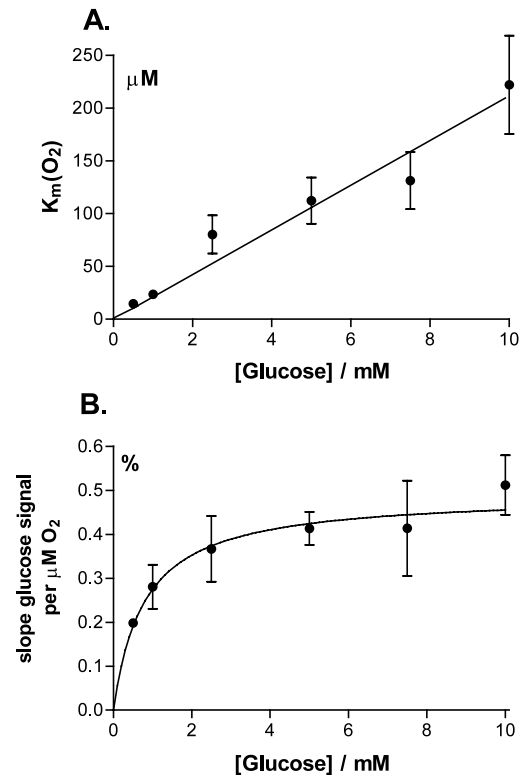


Fig. 3. (A) A plot of the average  $K_m(O_2)$  values vs. glucose concentration, obtained using Eq. (4) and glucose–oxygen correlation data similar to that shown in Fig. 1B, and linear regression analysis ( $R^2 > 0.96$ ), showing the increased sensitivity of the biosensor signal to solution  $pO_2$  as the concentration of glucose was increased. (B) The average percentage  $O_2$  interference in the glucose signals at 50  $\mu M$   $O_2$  per micromolar change in  $O_2$  concentration ( $S_{\%}$ ), obtained by averaging the tangential slopes (at 50  $\mu M$   $O_2$ ) of plots similar to those in Fig. 1B. A hyperbolic curve was fitted to allow  $S_{\%}$  to approach zero as the concentration of glucose approaches zero (see text for discussion).

zero. Unfortunately, because of the stirring needed for the determination of  $O_2$  and the long time needed to remove  $O_2$  from the system ( $\sim 3$  h, Fig. 1), sufficiently stable glucose signals for concentrations below 0.5 mM were not achievable in vitro. However, one can argue that as the concentration of glucose, and therefore the turnover of enzyme (Eq. (1)), approach zero, the Pt/PPD/GOx signal would be the same for 49 and 51  $\mu M$   $O_2$ ; i.e.,  $S_{\%}$  should also approach zero. Therefore the hyperbolic fit is the better one, a conclusion supported by the finding that  $K_m(O_2)$  approaches zero under these conditions (Fig. 3A). Using this fit, the extrapolated value of  $S_{\%}$  for 350  $\mu M$  glucose was calculated as 0.14% per  $\mu M$   $O_2$ . This suggests that increases in brain  $pO_2$  would have negligible impact ( $< 0.14\%$  per  $\mu M$   $O_2$ ) on the glucose signal recorded in brain ECF and that decreases in  $pO_2$  would only be significant if the tissue  $O_2$  levels became severely depleted. The non-linearity of these functions, however, preclude the use of a single number to define the interference of  $O_2$  in measuring

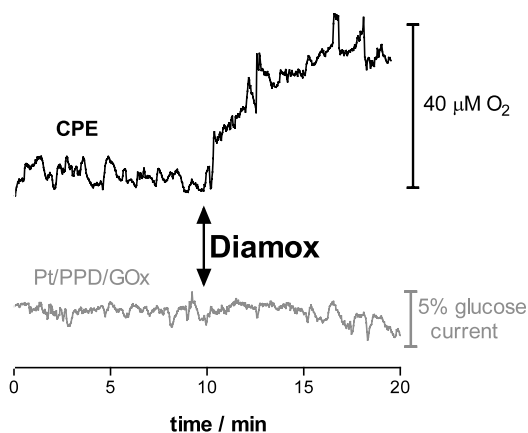


Fig. 4. The effect of systemic administration of the carbonic anhydrase inhibitor (Diamox, 50 mg/kg, i.p.) on the  $O_2$  and glucose signals monitored simultaneously in vivo in rat brain (striatum). The  $O_2$  current (black) was recorded using DPA with a CPE in the left striatum while glucose (grey) was detected using amperometry at +700 mV with a Pt/PPD/GOx electrode in the right striatum.

glucose, and so this conclusion was tested by monitoring the Pt/PPD/GOx glucose signal in brain ECF and manipulation brain  $pO_2$ .

### 3.3. Oxygen-sensitivity of Pt/PPD/GOx responses to glucose in vivo

The carbonic anhydrase inhibitor acetazolamide (Diamox) administered systemically has been shown to increase  $pO_2$  in brain ECF (Clark and Lyons, 1965). Fig. 4 shows the effect of this drug (50 mg/kg, i.p.) on the glucose and  $O_2$  signals recorded simultaneously in rat striatum. As expected, Diamox increased  $O_2$  levels in the ECF. Calibration of the CPEs recovered after the experiments allowed us to estimate this increase as  $40 \pm 12 \mu M$  ( $n = 5$ ,  $P < 0.03$ ) 10 min after the injection, which is similar to the increase observed following mild stress (Lowry et al., 1996). There was no corresponding change in the glucose signal at this time:  $9.7 \pm 3.6$  nA before and  $8.9 \pm 2.9$  nA after injection;  $n = 5$ ,  $P > 0.35$ , paired  $t$ -test; see Fig. 4. Thus, although the estimated concentration of brain extracellular  $O_2$  is relatively low

at approximately 50  $\mu M$  (Nair et al., 1987; Murr et al., 1994), significant increases in its concentration did not affect the glucose current, supporting the  $O_2$  interference analysis presented in Section 3.2.

A more critical test of the reliability of the Pt/PPD/GOx sensors to monitor glucose in the presence of fluctuating  $pO_2$  is the effect on the glucose signal of decreasing ECF  $pO_2$ . Anaesthetic doses of Ketamine have been shown recently to cause biphasic changes in brain ECF  $pO_2$  (Lowry and Fillenz, 2001), and so we used this drug to investigate further the  $O_2$  interference question. Ketamine (200 mg/kg, i.p., see Fig. 5) caused a decrease in the initial phase of  $32 \pm 9 \mu M$  ( $n = 7$ ,  $P < 0.02$ ) in striatal  $O_2$  20 min after the injection. At that time, there was no significant change in the biosensor signal:  $10.6 \pm 2.7$  nA before and  $10.6 \pm 2.8$  nA 20 min after injection ( $n = 6$ ,  $P > 0.99$ , paired  $t$ -test). Thereafter, the glucose and  $O_2$  signals changed in opposite directions (Fig. 5), reflecting physiological phenomena and demonstrating further the independence of the glucose signal from oxygen interference. These findings suggest that even large decreases in  $pO_2$  ( $\sim 60\%$  based on present estimates of average  $pO_2$  values and post-implant calibration data) do not affect the ability of Pt/PPD/GOx electrodes to monitor glucose reliably in brain ECF in vivo.

## 4. Conclusions

The in vitro and in vivo studies on the  $O_2$  dependence of Pt/PPD/GOx sensors reported here are internally consistent and indicate that these biosensors can be used reliably in brain ECF to monitor glucose even when there are large fluctuations in  $pO_2$ . The main factor enabling this functionality is the relatively low concentration of glucose in brain ECF compared with other tissues, such as blood and subcutaneous fat where the levels of glucose could prevent its reliable quantification using these devices. It remains to be seen whether the  $O_2$  insensitivity of Pt/PPD/GOx electrodes in the brain extends to lower (ischemic) levels of  $pO_2$ , and to

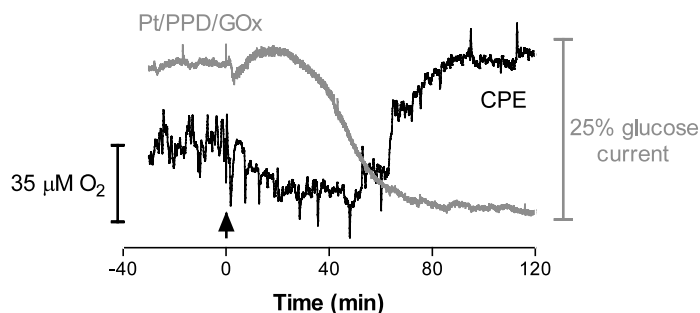


Fig. 5. The effect of systemic administration of an anaesthetic dose of Ketamine (200 mg/kg, i.p. at the arrow) on the  $O_2$  and glucose signals monitored simultaneously in vivo in rat brain. The  $O_2$  current (black) was recorded using DPA with a CPE in the left striatum while glucose (grey) was detected using amperometry at +700 mV with a Pt/PPD/GOx electrode in the right striatum.

electrodes incorporating other enzymes, such as glutamate and lactate oxidases.

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